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Unveiling the transcriptional control of pleiotropic drug resistance in *Saccharomyces cerevisiae*: Contributions of André Goffeau and his group

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Abstract

Studies in the yeast *Saccharomyces cerevisiae* have provided much of the basic detail underlying the organization and regulation of multiple or pleiotropic drug resistance gene network in eukaryotic microbes. As with many aspects of yeast biology, the initial observations that drove the eventual molecular characterization of multidrug resistance gene were provided by genetics. This review focuses on contributions from the laboratory of Dr. André Goffeau that uncovered key aspects of the transcriptional regulation of these multidrug resistance genes. André's group made many seminal discoveries that helped lead to the current picture we have of how eukaryotic microbes respond to and deal with a variety of antifungal agents. The importance of the transcriptional contribution to antifungal drugs is illustrated by the large number of drug resistant mutants found in several yeast species that lead to increased activity of transcriptional regulators. The characterization of the *S. cerevisiae PDR1* gene by the Goffeau group provided the first molecular basis explaining the link between this hyperactive transcription factor and drug resistance.

Keywords

Pleiotropic drug resistance; transcription; *Saccharomyces cerevisiae*; PDR1; PDR5

Introduction

Multidrug resistance is a problematic phenotype in many microorganisms but especially in the eukaryotic genera. The remarkable functional conservation between fungi and mammalian cells has been a boon to experimental biology but a serious challenge to development of fungal-specific chemotherapeutics (recently reviewed in (Fisher, Hawkins, Sanglard, & Gurr, 2018)). The primary class of antifungal drugs used in the clinic are the azole drugs which serve as the gold standard for chemotherapy in fungal infections (reviewed in (Denning & Hope, 2010)). This is also the only antifungal drug that can be delivered via an oral route, avoiding hospitalization.

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While azole drugs have been successfully employed since their introduction in the 1980s, this same success has led to the generation of resistant organisms. The two primary fungal pathogens are the *Candida* species: *Candida albicans* and *Candida glabrata*. Azole resistant isolates of both these species have been reported with the majority of these mapping to genes encoding transcriptional regulatory proteins (Morschhauser, 2010; Paul & Moye-Rowley, 2014; Sanglard, Coste, & Ferrari, 2009). This illustrates the central importance of gene regulation in acquisition of drug resistance. The mechanism of azole resistance in these pathogens is closely related to that seen for multiple drug resistance in *S. cerevisiae*. In fact, much of what we currently know of azole resistance in the *Candida* species is directly analogous to earlier findings from *S. cerevisiae* mutants that develop a multiple or pleiotropic drug resistant (Pdr) phenotype.

The goal of this review is to highlight the contributions to the understanding of the transcriptional basis of the Pdr phenotype that were made in the laboratory of André Goffeau during the late 1980s to early 2000s (Figure 1 and 2). This work set the stage for later studies directly in fungal pathogens which continue to this day.

***PDR1* is a Cys₆Zn₂ cluster-containing transcription factor**

The foundational observation that uncovered the basis for Pdr in *S. cerevisiae* came from the isolation of mutants that were resistant to a wide range of different drugs. These drugs exerted toxicity using different mechanisms and were chemically unrelated. Study of these mutants, especially by the group of Gerry Rank in Canada, determined that a single gene segregating 2:2 in tetrads called *PDR1* was responsible for these multiple resistant phenotypes. However, nothing was known of the molecular basis of resistance (Colson, Goffeau, Briquet, Weigel, & Mattoon, 1974; Rank & Bech-Hansen, 1973). The Goffeau group became interested in this locus as it mapped quite closely to the gene encoding the plasma membrane ATPase Pma1 that had been studied in Louvain-La-Neuve for many years and genetically mapped and characterized by Stanislaw Ulaszewski (Delhez, Dufour, Thines, & Goffeau, 1977; Ulaszewski, Balzi, & Goffeau, 1987). However, the connection between the Pma1 protein and the *PDR1* was unknown.

This mystery was solved when Elisabetta Balzi and colleagues, using a chromosome walking approach, identified a large cosmid clone that contained the chromosomal region corresponding to *PDR1* in 1987 (Balzi, Chen, Ulaszewski, Capieaux, & Goffeau, 1987). Subcloning and DNA sequence analysis provided the first determination that this gene encoded a transcriptional regulatory protein containing a DNA-binding domain corresponding to a Cys₆Zn₂ zinc cluster domain. This type of DNA-binding domain was shared with the galactose catabolic gene activator protein Gal4 as well as other fungal transcription factors (reviewed in (MacPherson, Larochelle, & Turcotte, 2006)). Use of a *pdr1* Δ URA3 disruption allele generated in the course of these experiments provided strong evidence that loss of *PDR1* from either drug hyper-resistant or wild-type strains produced a highly drug susceptible derivative.

These data argued that Pdr1 was likely a positive regulator of drug resistance as its loss rendered cells extremely sensitive to drug challenge. It is interesting to note that this was not what André had expected (or in fact hoped) as the *PDR1* locus was linked to the gene

encoding the plasma membrane ATPase, another of André's scientific interests. He had thought that the broad-spectrum drug resistance linked to *PDR1* might be due to this locus being allelic with *PMA1* (Goffeau, 2004) with *pma1* mutants affecting drug uptake. While it was not the membrane protein he had expected, the discovery of *PDR1* led to important understanding of the nature of the Pdr phenotype in *S. cerevisiae*.

Pdr1 controls *PDR5* and other ABC transporter genes

Having determined that Pdr1 was a transcription factor, the next question was: what were the regulated genes that directly conferred drug resistance? A model was proposed that Pdr1 might control the transcription of genes encoding proteins that determined the permeability barrier of the yeast cell (Balzi & Goffeau, 1991). The early 1990s produced the next important step forward in dissection of the role of Pdr1 in drug resistance. Although *PMA1* did not play a direct role in the Pdr phenotype, another critically important membrane transporter did. This transporter, encoded by the *PDR5* gene, was originally identified in a high copy screen searching for genes that could elevate resistance to two different inhibitors of growth, cycloheximide and the herbicide sulfometuron methyl (Leppert et al., 1990). This screen conducted by John Golin led to the identification of both *PDR5* and the basic region-leucine zipper transcription factor *YAPI* (originally called *PDR4*). As Moye-Rowley had cloned *YAPI* earlier via a biochemical approach (Moye-Rowley, Harshman, & Parker, 1989), this led to an excited series of FAX messages between the Moye-Rowley and Goffeau groups (this was pre-e-mail!) to arrange exchanges of clones and sequences (Figure 3). This had the benefit of connecting the Goffeau and Moye-Rowley labs in a productive collaboration that led to several shared publications.

A collaborative effort between the Golin and Goffeau labs used genetic analyses to determine that gain-of-function (GOF) forms of *PDR1* required the presence of *PDR5* for resistance to cycloheximide but interestingly not for sulfometuron methyl (Meyers et al., 1992). Additionally, northern blot experiments determined that *PDR5* mRNA was overproduced in GOF *PDR1* backgrounds.

These data focused attention on the *PDR5* gene and led to the determination of its DNA sequence by Elisabetta Balzi and others in 1994 (Balzi, Wang, Leterme, Van Dyck, & Goffeau, 1994). Independently, the labs of Karl Kuchler and Tokichi Miyakawa cloned and sequenced *PDR5* (Bissinger & Kuchler, 1994; Hirata, Yano, Miyahara, & Miyakawa, 1994). These studies led to the discovery that *PDR5* encoded an ATP-binding cassette (ABC) transporter. The Pdr5 protein was shown to be overexpressed in the plasma membrane of *pdr1* mutants (Balzi et al., 1994) and was characterized as a nucleotide triphosphatase sensitive to a variety of hydrophobic compounds (Decottignies, Kolaczowski, Balzi, & Goffeau, 1994). This was a major step forward in providing a clear basis for the Pdr phenotype as it was well-established in mammalian cells that overproduction of the Mdr1 ABC transporter was strongly associated with multidrug resistance in cancer cells (reviewed in (Pastan & Gottesman, 1991)).

Having established that Pdr1-dependent transcriptional activation of *PDR5* was required for a subset of the drug resistance detected in a GOF *PDR1* background, Anabelle Decottignies went on to demonstrate that Pdr1 also controlled the expression of another ABC transporter-

encoding gene designated *SNQ2* (Decottignies et al., 1995). This link between Pdr1 and *SNQ2* was confirmed by studies from the Kuchler lab (Mahe et al., 1996). Coupling these data with the demonstration that another ABC transporter-encoding gene called *YOR1* was both regulated by Pdr1 and required for resistance to the mitochondrial ATPase inhibitor oligomycin (Katzmann et al., 1995), a clear picture emerged for the basic outline of the Pdr network of genes. Pdr1 served as a central regulator for a suite of different ABC transporter genes with each of these transporters conferring a subset of the overall drug resistance profile associated with GOF forms of *PDR1*.

It was clear that GOF forms of Pdr1 were hyperactive but the structure of these alleles was unknown and many of these were recovered from widely disparate genetic backgrounds. To resolve both of these shortcomings, Elvira Carvajal cloned 3 different GOF alleles of *PDR1* into a low-copy-number plasmid, sequenced each in its entirety and then compared their function in the same genetic background (Carvajal, van den Hazel, Cybularz-Kolaczowska, Balzi, & Goffeau, 1997). Single amino acid replacements were found to be sufficient to cause the resulting Pdr1 derivative to drive elevated drug resistance and *PDR5* transcription.

The Pdr1-*PDR5* transcription regulatory circuit also allowed the identification and characterization of similar ABC transporters and a related transcriptional induction responsible for multidrug resistance in *Aspergillus nidulans*, in the frame of a collaboration between the Goffeau and M. De Waard labs (Del Sorbo et al., 1997).

Similarly, the Pdr1-*PDR5* system served as a developmental platform for drug screening. The use of *PDR1* GOF mutations and the *PDR5* promoter in appropriate *S. cerevisiae* strains deleted for multiple ABC transporter-encoding loci allowed high-level overexpression of Pdr5p, Snq2p or Yor1p. These overexpressed proteins exhibited ATPase activity *in vitro* and conferred multidrug resistance *in vivo*. This latter property was used for screening specific inhibitors of ABC transporters from multiple sources (Rogers et al., 2001).

Pdr genes extend beyond ABC transporters

While the majority of Pdr1 target genes that were characterized initially all encoded ABC transporters, it was clear there were other regulatory targets. The first of these was the *PDR3* encoding a transcription factor closely related to Pdr1. The original genetic localization of *PDR3* was carried out by Julius Subik and Stan Ulaszewski who mapped this gene to the left arm of chromosome II as a multidrug resistant allele with phenotypes similar to those conferred by GOF forms of *PDR1* (Subik, Ulaszewski, & Goffeau, 1986). Work from Claude Jacq's laboratory demonstrated that *PDR3* encoded a transcription factor showing high sequence similarity with Pdr1 (Delaveau, Delahodde, Carvajal, Subik, & Jacq, 1994). *PDR3* was found to be autoregulated via two Pdr response elements (PDREs) present in its promoter (Delahodde, Delaveau, & Jacq, 1995). Additional studies by the Jacq and Subik labs discovered that point mutations in *PDR3* similar to those in *PDR1* also led to the acquisition of a GOF phenotype for this related factor (Nourani, Papajova, Delahodde, Jacq, & Subik, 1997; Simonics et al., 2000).

This wealth of knowledge allowed the first outline of the Pdr network to be proposed (Balzi & Goffeau, 1995). Later, the definitive membership in the Pdr regulon came from a

microarray study carried out by the Goffeau lab with the collaboration of Claude Jacq and Pat Brown (DeRisi et al., 2000). This microarray experiment represented one of the earliest examples of this new technology and provided invaluable new insight into the list of genes that were responsive to GOF forms of both *PDR1* and *PDR3*. The 26 genes that were found to be upregulated in the presence of the hyperactive Pdr1/3 factors encoded 6 different classes of proteins including 4 ABC transporters, another drug efflux transporter class (Tpo1 is a major facilitator superfamily member) with the rest being involved in lipid biosynthesis, stress response, cell wall production or of an unknown function. While this experiment was restricted to expression measurements only, additional studies confirmed the membership of these new genes in the Pdr regulon.

Verification of the new target genes identified by this microarray study came from a collaborative study on the *IPT1* gene between the Goffeau and Moye-Rowley labs. *IPT1* had been identified as a Pdr1 target gene in the microarray study and work from Bob Dickson's lab demonstrated that this locus encoded the last step in biosynthesis of sphingolipids (Dickson, Nagiec, Wells, Nagiec, & Lester, 1997). *IPT1* was found to contain a single PDRE in its promoter and to influence biosynthesis of the major plasma membrane sphingolipid species mannosyl diphosphorylinositol ceramide by the work of Stephan Schorling (Hallstrom et al., 2001). Later studies demonstrated that several earlier steps in the pathway of sphingolipid biosynthesis were also under control of Pdr1/Pdr3 (Kolaczowski, Kolaczowska, Gaigg, Schneider, & Moye-Rowley, 2004).

Studies from Maria do Valle Matta and Bart van den Hazel provided evidence that both the *TPO1* (MFS drug efflux transporter) (do Valle Matta, Jonniaux, Balzi, Goffeau, & van den Hazel, 2001) and *PDR16* (phospholipid transfer protein) (van den Hazel et al., 1999) genes were targets within the Pdr regulon. Together, these studies provided the first picture of the genes that were regulated in the context of the genomic response by key regulators of drug resistance in any eukaryotic organism.

Conclusions

The early state of understanding of the Pdr phenotype in yeast was limited to the identification of mutant alleles of *PDR1* and *PDR3* that had the interesting features of elevating resistance to a range of different toxic drugs. The isolation and characterization of the *PDR1* gene changed the fundamental nature of models underlying this multidrug resistance phenotype; first in *S. cerevisiae* and then in the *Candida* species (also initially in the Goffeau lab by Rajendra Prasad (Prasad, Dewergifosse, Goffeau, & Balzi, 1995)). The integral roles played by ABC transporter proteins were entirely consistent with those seen earlier in animal cells but the bases of the increased expression of the fungal and mammalian transporter-encoding genes were different. Animal cells typically amplified the copy number of these genes (see (Roninson, 1992) for an early review) while fungal cells employed GOF transcription factors to elevate the transcription of the single copy loci (reviewed in (Balzi & Goffeau, 1991; Morschhauser, 2010)).

A second major contribution was the expansion of the target gene suite for the Pdr transcription factors. The initial picture of the target genes suggested that primarily loci

encoding ABC transporters were controlled by Pdr1 (and Pdr3). The microarray experiments on GOF forms of both *PDR1* and *PDR3* provided clear indication that only a fraction of these transcriptionally-controlled target genes corresponded to ABC transporters (DeRisi et al., 2000; Devaux et al., 2001). These genes include loci involved in lipid biosynthesis, seven transmembrane domain-containing proteins, transcription factors and a number of proteins with functions that remain uncertain even now. The evolutionarily closely related pathogen *Candida glabrata* shares both a Pdr1 factor and most of these genes (Vermitsky et al., 2006). While the more divergent *Candida albicans* presents an interesting case. *C. albicans* still co-regulates many of these same targets but does so using transcription factors (Tac1, Mrr1) that are not closely related to either Pdr1 or Pdr3 (Coste, Karababa, Ischer, Bille, & Sanglard, 2004; Morschhauser et al., 2007). These data suggest that *C. albicans* may have rewired the Pdr regulon to respond to very different signals than those detected by *S. cerevisiae* and *C. glabrata*.

While the scientific contributions driven by André Goffeau were outstanding (as we hope this review partially illustrates), his roles as a leader in the laboratory and a highly collaborative investigator were even more impactful. We mourn his loss but remember our good fortune in having had the pleasure to work with him not only as a mentor and a colleague but also a friend.

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Figure 1.
Andre Goffeau's group in Louvain-la-Neuve circa 1986.

Figure 2.
Andre Goffeau's group in Louvain-la-Neuve circa 1997, 10 years after the cloning of *PDR1*.

Figure 3.
André Goffeau and Scott Moye-Rowley at the Yeast Genetics and Molecular Biology Meeting in 2008 (Toronto, Canada)